Multiple molecular mechanisms for a single GABA_A mutation in epilepsy

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ABSTRACT

Objective: To understand the molecular basis and differential penetrance of febrile seizures and absence seizures in patients with the $\gamma 2(R43Q)$ GABA_A receptor mutation.

Methods: Spike-and-wave discharges and thermal seizure susceptibility were measured in heterozygous GABA_A $\gamma 2$ knock-out and GABA_A $\gamma 2(R43Q)$ knock-in mice models crossed to different mouse strains.

Results: By comparing the GABA_A $\gamma 2$ knock-out with the GABA_A $\gamma 2(R43Q)$ knock-in mouse model we show that haploinsufficiency underlies the genesis of absence seizures but cannot account for the thermal seizure susceptibility. Additionally, while the expression of the absence seizure phenotype was very sensitive to mouse background genetics, the thermal seizure phenotype was not.

Conclusions: Our results show that a single gene mutation can cause distinct seizure phenotypes through independent molecular mechanisms. A lack of effect of genetic background on thermal seizure susceptibility is consistent with the higher penetrance of febrile seizures compared to absence seizures seen in family members with the mutation. These mouse studies help to provide a conceptual framework within which clinical heterogeneity seen in genetic epilepsy can be explained. *Neurology*® 2013;80:1003-1008

GLOSSARY

SWD = spike-and-wave discharges.

Family members harboring the GABA_A $\gamma 2(R43Q)$ mutation display multiple seizure types, incomplete penetrance, and variable seizure severity,^{1,2} a common feature of genetic epilepsies.³ To predict clinical outcomes for patients based on their personal genomes, it is important that we develop a conceptual framework explaining the genetic and molecular basis of heterogeneity. Animal models of epilepsy, based on human mutations, provide a means of investigating this. The knock-in mouse model based on the GABA_A $\gamma 2(R43Q)$ mutation recapitulates the 2 major phenotypes seen in family members, including febrile seizures and typical absence seizures.^{4,5} However, the molecular mechanisms causing epilepsy in this model are unclear. In vitro studies suggest that the deficit could be through haploinsufficiency (loss-of-function) or a dominant impact of the mutated protein.⁶ To investigate this, we compared the seizure phenotypes of heterozygous *Gabrg2* knock-out mice with those of knock-in mice.

Penetrance is relatively low for absence seizures in the GABA_A $\gamma 2(R43Q)$ family.^{1,2} By back-crossing the GABA_A $\gamma 2(R43Q)$ knock-in mouse to strains with different seizure susceptibility, we have demonstrated that the spike-wave phenotype requires additional susceptibility alleles for full expression,^{5,7} potentially explaining the low penetrance of absence in the family. In contrast, febrile seizures segregate as a more highly penetrant autosomal dominant trait, which suggests that background genetics have less impact.^{1,2} Here we investigate the influence of genetic background on thermal seizure and spike-wave susceptibility in the knock-in mouse model.

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METHODS Mice. All experiments were approved by the Animal Ethics Committee at the Florey Institute of Neuroscience and Mental Health (09-046). Genotyping of the GABA_A $\gamma 2$ (R43Q) knock-in mouse model and the GABA_A $\gamma 2^{+/-}$ heterozygous knock-out mice was done at P12 using PCR-based methods previously described.^{5,8} Mice were used between the ages P35–P40 for electrocorticogram recordings. For thermogenic stress assay mice were used at P16–P17. Animals were crossed to >N9 into a DBA/2J and to >N20 for C57BL/6J background.

Electrocorticogram recordings. For electrocorticograms, surgeries were performed as previously described.⁵ Mice were anesthetized with 1%–3% isoflurane and 2 epidural silver “ball” electrodes implanted on each hemisphere of the skull. Electrodes were placed 3 mm lateral of the midline and 0.5 mm caudal from bregma. A ground electrode was placed 2.5 mm rostral from bregma and 0.5 mm lateral from the midline. Mice were allowed to recover for at least 24 hours after surgery. Electrocorticograms were continuously recorded in freely moving mice for a 4-hour period during daylight hours following a standard 30-minute habituation period. Total time in seizure was defined as the percent time each animal spent having spike-and-wave discharges (SWD). Data from male and female mice were pooled in this study. Signals were bandpass filtered at 0.1 to 200 Hz and sampled at 1 kHz using Powerlab 16/30 (ADInstruments Pty. Ltd., Sydney, NSW, Australia). In a subset of mice electrocorticogram recordings were simultaneously recorded with video to enable the scoring of behavioral arrest. Statistical comparison was made using an unpaired *t* test (GraphPad Prism, La Jolla, CA).

Thermogenic seizure testing. P16–P17 mice were placed in a container heated to constant 42°C and the time to first tonic-clonic seizure recorded based on methods previously described.⁹ Mice were killed immediately after the first observed seizure to comply with our animal ethics approval. Survival curves were analyzed using the Mantel-Cox method (GraphPad Prism). There was no difference in latency to seizure between wild-type littermates of the knock-in and knock-out models (*p* = 0.4) and these animals were combined for the control.

Drug studies. Ethosuximide was dissolved in saline to a final concentration of 20 mg/mL delivered via IP injection at 0.01 mL/g. Electrocorticogram recordings were done for 150 minutes prior to injection and recorded for a further 135 minutes following injection. SWDs were counted in a 120-minute epoch prior to injection and compared to a 120-minute epoch following injection. Statistical comparison was made using paired *t* test (GraphPad Prism).

qPCR. Total RNA from cortex was extracted with TRizol (Invitrogen, Carlsbad, CA) and purified and concentrated using an RNeasy Kit according to manufacturer's instructions (Qiagen, Hilden, Germany). RNA quality was assessed by spectrophotometry for 260/280 (all were 2.0–2.05) and 260/230 ratios (all above 1.8). A total of 1 μ g of total RNA was reverse transcribed to cDNA in a 20- μ L reaction using random hexamer primers and a Roche (Branchburg, NJ) High Fidelity Transcription Kit. qPCR of 10 ng cDNA from 4 (knock-out mouse) and 7 (knock-in mouse) littermate pairs was performed using a SYBR Green Master Mix (Fermentas Life Sciences, Vilnius, Lithuania) and primers to exon 8. Exon 8 is deleted in the mutant allele, causing a frameshift that curtails translation after exon 7.⁹ Primers for the exon 8 R allele were as follows: forward 5' tctctgcccaaggctccta 3' and reverse 5' acaaaataatgcagggtgcc 3', and for GADPH (endogenous control) forward 5' ggtggtctctctgactcaaca 3' and reverse 5' gttgctgtagc-caaattcgtgtg 3'. The R allele in the knock-in mice was assayed using primers forward 5' gtcattctaacaacctgtggaa 3' and reverse 5'

ccaatgctgttcacatacatatctgt 3' and a hydrolysis probe 5' tatgacaa-caacttcgacctgacatagg 3' to exon 2. The knock-in mutation changes 5 bp in exon 2. The level of R allele transcript in the knock-out heterozygote was calculated relative to that of the wild-type littermate and corrected for amplification efficiencies (Pfaffl, 2001 REST software, Qiagen). Comparison between genotypes was made using the Mann-Whitney nonparametric test (GraphPad Prism).

RESULTS Haploinsufficiency is the underlying molecular basis of the absence seizure phenotype. In this study we compare the seizure phenotypes of the GABA_A $\gamma 2$ (R43Q) knock-in mouse model with that of GABA_A $\gamma 2^{+/-}$ heterozygous knock-out mice,¹⁰ both backcrossed to the same seizure-susceptible DBA/2J strain (DBA).¹¹ The DBA-GABA_A $\gamma 2$ (R43Q) mice display SWD on electrocorticograms associated with behavioral arrest,⁵ consistent with absence seizures seen in patients (figure 1, A and B). Further analyses of electrocorticograms showed that the DBA-GABA_A $\gamma 2^{+/-}$ knock-out model spent a similar amount of time in SWD seizures as the DBA-GABA_A $\gamma 2$ (R43Q) knock-in mouse (figure 1, A and B). Moreover, SWDs in the GABA_A $\gamma 2^{+/-}$ were sensitive to the first-line anti-absence seizure drug ethosuximide (200 mg/kg, 2.08 ± 0.4 vs 0.31 ± 0.08 , *n* = 5, *p* < 0.001), similar to the ~80% reduction in SWDs reported for the DBA-GABA_A $\gamma 2$ (R43Q) mouse.⁵ These findings suggest that haploinsufficiency is the underlying molecular basis of the absence seizure phenotype in the knock-in as well as the knock-out model.

Haploinsufficiency cannot account for the febrile seizure phenotype. Febrile seizures are a feature of the GABA_A $\gamma 2$ (R43Q) family.² To test the thermogenic seizure susceptibility of the mice models, we subjected young mice to a constant 42°C environment and measured the latency to the first tonic-clonic seizure.⁹ In this assay, DBA-GABA_A $\gamma 2$ (R43Q) mice developed seizures with a lower latency than wild-type controls, suggestive of heightened febrile seizure susceptibility as seen in the patients (figure 1C, *p* < 0.005). Importantly, the time to seizure of the DBA-GABA_A $\gamma 2^{+/-}$ mouse was indistinguishable from that of wild-type (figure 1C, *p* = 0.95), demonstrating that although the knock-out model shares the absence seizure phenotype it does not share the thermal seizure phenotype with the DBA-GABA_A $\gamma 2$ (R43Q) knock-in model.

Similar levels of the wild-type allele in the 2 mouse models. The mRNA levels of the wild-type (R) allele determined by qPCR were the same for both the GABA_A $\gamma 2$ (R43Q) heterozygous knock-in and GABA_A $\gamma 2^{+/-}$ knock-out models when compared with their wild-type littermates (0.57 ± 0.05 vs 0.59 ± 0.09 , *n* = 7 pairs and 4 pairs, respectively, *p* = 0.83). This suggests that the major genetic difference between the 2 models is in haploinsufficiency and the missense mutation.

Figure 1 Two distinct molecular mechanisms underlie the 2 major epilepsy phenotypes caused by the GABA_A $\gamma 2$ (R43Q) mutation

(A) Electrocorticogram recordings from DBA/J2 GABA_A $\gamma 2$ (R43Q) (DBA- $\gamma 2^{R/Q}$) and DBA/J2 GABA_A $\gamma 2^{+/-}$ (DBA- $\gamma 2^{+/-}$) mice illustrating spontaneous spike-and-wave discharges (SWDs). Upward and downward pointing arrows signify onset and offset of behavioral arrest. The asterisk indicates an event on the expanded time scale illustrated below each trace. Scale (x) bars represent 4 s and 1.5 s for top and bottom traces, respectively. (B) Average total time spent in SWD for the wild-type (DBA- $\gamma 2^{R/R}$ [n = 10]), DBA- $\gamma 2^{R/Q}$ [n = 8], and DBA- $\gamma 2^{+/-}$ [n = 5]) mouse models. * $p < 0.005$ as compared to the DBA- $\gamma 2^{R/Q}$ or DBA- $\gamma 2^{+/-}$ models. (C) Assessment of susceptibility to thermal-induced tonic-clonic seizures (DBA- $\gamma 2^{R/Q}$; n = 13, DBA- $\gamma 2^{R/R}$; n = 20, DBA- $\gamma 2^{+/-}$; n = 12). Mantel-Cox method was used for statistical comparison of survival curves (DBA- $\gamma 2^{R/Q}$ vs DBA- $\gamma 2^{R/R}$ $p < 0.05$, DBA- $\gamma 2^{+/-}$ vs WT $p = 0.95$, DBA- $\gamma 2^{+/-}$ vs DBA- $\gamma 2^{R/Q}$ $p < 0.05$).

Differential sensitivity of the 2 seizure phenotypes to background genetics. We have previously demonstrated that SWD expression is sensitive to background genetics by backcrossing the GABA_A $\gamma 2$ (R43Q) knock-in model to 2 mouse strains.^{5,7} Here we confirm this by showing that the GABA_A $\gamma 2$ (R43Q) knock-in backcrossed into the C57BL/6J strain (C57) does not have a SWD phenotype (figure 2, A and B). To test the

sensitivity of the febrile seizure phenotype to genetic background, we exposed C57-GABA_A $\gamma 2$ (R43Q) and DBA-GABA_A $\gamma 2$ (R43Q) knock-in mice and their wild-type controls to thermogenic stress as described above. Both strains of knock-in mice developed seizures sooner than their wild-type controls (figure 2C, $p < 0.005$), suggesting that they both have the febrile seizure phenotype.

Figure 2 Spike-and-wave discharges but not thermogenic seizures of the GABA_A $\gamma 2(R43Q)$ mice are sensitive to background genetics consistent with penetrance seen in the family

(A) Electrocorticogram recordings from GABA_A $\gamma 2(R43Q)$ mice backcrossed into 2 different background strains. Spontaneous spike-and-wave discharges (SWDs) are observed in the DBA- $\gamma 2^{R/Q}$ knock-in but not the C57BL/6J GABA_A $\gamma 2(R43Q)$ knock-in strain (C57- $\gamma 2^{R/Q}$). (B) Summary histogram of SWD events recorded from the DBA- $\gamma 2^{R/Q}$ ($n = 40$) and C57- $\gamma 2^{R/Q}$ ($n = 10$) strains. (C) Assessment of susceptibility to thermal-induced tonic-clonic seizures between knock-in mice and their wild-type controls (DBA- $\gamma 2^{R/Q}$ [$n = 16$], DBA $\gamma 2^{R/R}$ [$n = 10$], C57- $\gamma 2^{R/Q}$ [$n = 13$], C57- $\gamma 2^{R/R}$ [$n = 12$]). Mantel-Cox method was used for statistical comparison of survival curves (DBA- $\gamma 2^{R/Q}$ vs DBA $\gamma 2^{R/R}$ $p = 0.021$, C57- $\gamma 2^{R/Q}$ vs C57- $\gamma 2^{R/R}$ $p = 0.003$, DBA- $\gamma 2^{R/Q}$ vs C57- $\gamma 2^{R/Q}$ $p = 0.543$, DBA $\gamma 2^{R/R}$ vs C57- $\gamma 2^{R/R}$ $p = 0.641$). (D) Table summarizing the seizure phenotype of each mouse model tested.

DISCUSSION Over the past decade, it has remained an open question as to whether the fundamental molecular deficit of the mutant $\gamma 2(R43Q)$ protein is due to haploinsufficiency or a dominant impact of the mutated protein, or both.⁶ We had assumed that one or another of these molecular mechanisms was the underlying basis of all epilepsy phenotypes seen in this family. In this study, we demonstrate that haploinsufficiency is able to account for the genesis of SWDs but cannot account for the thermal seizure

susceptibility. Therefore, thermal seizure susceptibility is governed by a different molecular mechanism, presumably as a consequence of an additional effect of the mutant $\gamma 2(R43Q)$ protein. Importantly, the current study provides a unifying explanation for in vitro data on the $\gamma 2(R43Q)$ mutation.¹²⁻¹⁵

There are clues in the literature as to what the downstream cellular mechanisms might be for each phenotype seen in the $\gamma 2(R43Q)$ family. Analysis of the binding of radioactive flumazenil, a competitive

antagonist at the benzodiazepine site that is dependent on the GABA_A receptor $\gamma 2$ subunit, indicates that the knock-in mice, knock-out mice, and the GABA_A $\gamma 2$ (R43Q) family members all demonstrate a similar degree of reduced binding consistent with simple haploinsufficiency in each case.^{4,16,17} One possible mechanism of spike-wave genesis in the knock-in and knock-out models may be that single channel conductance of GABA_A receptors is lower. In the GABA_A $\gamma 2^{+/-}$ mouse there is a significant increase in the proportion of smaller conductance amplitude measured from hippocampal and dorsal root ganglia neurons.¹⁸ Also consistent with this is a reduction in phasic GABA_A receptor-mediated inhibition in the cortex of the GABA_A $\gamma 2$ (R43Q) knock-in mouse model.⁵ In contrast, the febrile seizure phenotype is likely to be caused by an interaction that results in an additional neuronal phenotype unique to the GABA_A $\gamma 2$ (R43Q) knock-in mouse. One interesting example proposed for this mutation is a reduction in the cell surface expression of the GABA_A $\alpha 5$ subunit by the mutated $\gamma 2$ (R43Q) protein.¹³ GABA_A $\alpha 5$ -containing subunits are found extrasynaptically with their activation resulting in a tonic GABA-mediated current in the hippocampus.¹⁹ Altered tonic inhibition has been associated with increased neuronal excitability and epilepsy.²⁰ Alternatively, differences in kinetics or altered sensitivity to modulation of any surface receptor containing mutated $\gamma 2$ subunit may contribute to febrile seizure susceptibility.^{12,14} Having a clear idea of the molecular start point for the basis of each seizure type is critical to how we interpret data aimed at understanding cellular mechanisms. Comparing molecular, cellular, and neuronal network deficits of the knock-in with the knock-out model will provide a powerful means of helping to isolate the precise disease mechanism for both seizure phenotypes in future studies.

Our mouse studies show that the absence phenotype can be dependent on genetic background, with knock-in C57BL/6J mice having no SWDs while the knock-in DBA/2J robustly expresses the phenotype⁷ and the febrile seizure phenotype occurs in both background strains. This is consistent with the clinical prediction made in the original family,² where the penetrance of these 2 primary seizure phenotypes is different in family members harboring the mutation, with febrile seizures in ~65% and absence seizures in ~20%. The pattern observed in the mouse models suggests that this increased penetrance in patients is, at least in part, due to a differential sensitivity of the 2 seizure phenotypes to genetic background. The genetic basis of SWD susceptibility in animal models is yet to be fully established. Several studies have implicated a region on chromosome 1 termed *Szs 1* as the primary basis of the different proconvulsant susceptibility of

the C57BL/6(B6) and DBA/2(D2) strains.^{21–23} More recently, using congenic mice strains, it was demonstrated that spontaneous SWD seizure susceptibility is sensitive to the *Szs 1* locus but that changes at this locus alone cannot account for the phenotype.²⁴ Our study implies that similar genetic principles occur in human populations and that mouse studies may provide the tools to begin to understand the complex heritability of common epilepsies.²⁵

We demonstrate that a single gene mutation can cause distinct seizure types through independent molecular mechanisms and that these seizure types differ in their sensitivity to genetic background. Febrile seizures are not a usual feature of childhood absence epilepsy and the occurrence of febrile seizures in the GABA_A $\gamma 2$ (R43Q) family is likely to be due to molecular mechanisms associated with specific cell biological changes caused by the $\gamma 2$ (R43Q) mutation. These observations now provide a framework to understand the apparently puzzling finding of phenotypic heterogeneity associated with a major dominant gene.

AUTHOR CONTRIBUTIONS

Dr. Reid: study concept and design, analysis and interpretation, drafting of manuscript. T. Kim: acquisition of data, analysis and interpretation, figures. Dr. Phillips: acquisition of data. Prof. Berkovic: critical revision of the manuscript for important intellectual content. Prof. Lüscher: study concept and design. A/Prof Petrou: study concept and design, analysis and interpretation, drafting of manuscript. All authors were involved in critically reading the manuscript.

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DISCLOSURE

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REFERENCES

1. Marini C, Harkin LA, Wallace RH, Mulley JC, Scheffer IE, Berkovic SF. Childhood absence epilepsy and febrile seizures: a family with a GABA(A) receptor mutation. *Brain* 2003;126:230–240.
2. Wallace RH, Marini C, Petrou S, et al. Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. *Nat Genet* 2001;28:49–52.
3. Helbig I, Scheffer IE, Mulley JC, Berkovic SF. Navigating the channels and beyond: unravelling the genetics of the epilepsies. *Lancet Neurol* 2008;7:231–245.
4. Hill EL, Hosie S, Mulligan RS, et al. Temperature elevation increases GABA(A) -mediated cortical inhibition in a mouse model of genetic epilepsy. *Epilepsia* 2011;52:179–184.

5. Tan HO, Reid CA, Single FN, et al. Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. *Proc Natl Acad Sci USA* 2007;104:17536–17541.
6. Reid CA, Berkovic SF, Petrou S. Mechanisms of human inherited epilepsies. *Prog Neurobiol* 2009;87:41–57.
7. Reid CA, Kim TH, Berkovic SF, Petrou S. Low blood glucose precipitates spike-and-wave activity in genetically predisposed animals. *Epilepsia* 2011;52:115–120.
8. Alldred MJ, Mulder-Rosi J, Lingenfelter SE, Chen G, Luscher B. Distinct gamma2 subunit domains mediate clustering and synaptic function of postsynaptic GABAA receptors and gephyrin. *J Neurosci* 2005;25:594–603.
9. Schuchmann S, Schmitz D, Rivera C, et al. Experimental febrile seizures are precipitated by a hyperthermia-induced respiratory alkalosis. *Nat Med* 2006;12:817–823.
10. Gunther U, Benson J, Benke D, et al. Benzodiazepine-insensitive mice generated by targeted disruption of the gamma 2 subunit gene of gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA* 1995;92:7749–7753.
11. Tan HO, Reid CA, Chiu C, Jones MV, Petrou S. Increased thalamic inhibition in the absence seizure prone DBA/2J mouse. *Epilepsia* 2008;49:921–925.
12. Bowser DN, Wagner DA, Czajkowski C, et al. Altered kinetics and benzodiazepine sensitivity of a GABAA receptor subunit mutation [gamma 2(R43Q)] found in human epilepsy. *Proc Natl Acad Sci USA* 2002;99:15170–15175.
13. Eugene E, Depienne C, Baulac S, et al. GABA(A) receptor gamma 2 subunit mutations linked to human epileptic syndromes differentially affect phasic and tonic inhibition. *J Neurosci* 2007;27:14108–14116.
14. Goldschen-Ohm MP, Wagner DA, Petrou S, Jones MV. An epilepsy-related region in the GABA(A) receptor mediates long-distance effects on GABA and benzodiazepine binding sites. *Mol Pharmacol* 2010;77:35–45.
15. Hales TG, Tang H, Bollan KA, et al. The epilepsy mutation, gamma2(R43Q) disrupts a highly conserved inter-subunit contact site, perturbing the biogenesis of GABAA receptors. *Mol Cell Neurosci* 2005;29:120–127.
16. Crestani F, Lorez M, Baer K, et al. Decreased GABAA-receptor clustering results in enhanced anxiety and a bias for threat cues. *Nat Neurosci* 1999;2:833–839.
17. Fedi M, Berkovic SF, Macdonell RA, Curatolo JM, Marini C, Reutens DC. Intracortical hyperexcitability in humans with a GABAA receptor mutation. *Cereb Cortex* 2008;18:664–669.
18. Lorez M, Benke D, Luscher B, Mohler H, Benson JA. Single-channel properties of neuronal GABAA receptors from mice lacking the 2 subunit. *J Physiol* 2000;527 pt 1:11–31.
19. Caraiscos VB, Elliott EM, You-Ten KE, et al. Tonic inhibition in mouse hippocampal CA1 pyramidal neurons is mediated by alpha5 subunit-containing gamma-aminobutyric acid type A receptors. *Proc Natl Acad Sci USA* 2004;101:3662–3667.
20. Brickley SG, Mody I. Extrasynaptic GABA(A) receptors: their function in the CNS and implications for disease. *Neuron* 2012;73:23–34.
21. Ferraro TN, Golden GT, Smith GG, et al. Quantitative genetic study of maximal electroshock seizure threshold in mice: evidence for a major seizure susceptibility locus on distal chromosome 1. *Genomics* 2001;75:35–42.
22. Ferraro TN, Golden GT, Smith GG, et al. Mapping loci for pentylenetetrazol-induced seizure susceptibility in mice. *J Neurosci* 1999;19:6733–6739.
23. Ferraro TN, Golden GT, Smith GG, et al. Mapping murine loci for seizure response to kainic acid. *Mamm Genome* 1997;8:200–208.
24. Bessaih T, de Yebenes EG, Kirkland K, et al. Quantitative trait locus on distal chromosome 1 regulates the occurrence of spontaneous spike-wave discharges in DBA/2 mice. *Epilepsia* 2012;53:1429–1435.
25. Frankel WN. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. *Trends Genet* 2009;25:361–367.

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